

PRESENTATION SUMMARY & POWERPOINT

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

Kathleen A. Fagerstone, Manager, Product Development Research Program,
USDA/APHIS/Wildlife Services, National Wildlife Research Center,
Fort Collins, Colorado

For most of the last century, federal and state wildlife conservation agencies in the United States have focused on conserving or increasing populations of many species of wildlife. Although wildlife abundance is desirable in most cases, populations of some species may reach undesirably high levels and cause either ecological damage or human-wildlife conflicts. Conflicts can include damage to agricultural commodities through depredations of livestock, crops, or forest resources. Buildings and other structures and properties can be damaged by nesting, burrowing, or feeding activities. Overabundant wildlife also can cause human health and safety issues, including wildlife-aircraft strikes and deer-vehicle collisions. There is increasing concern about the potential for wildlife disease transmission to humans and livestock (e.g., Lyme disease, tuberculosis, brucellosis, pseudorabies, West Nile virus, chronic wasting disease, avian influenza). Stray or feral animals such as cats, dogs and pigs can transmit disease and cause environmental damage. Many of the problems associated with overabundant wildlife occur in areas recently converted by suburban development or in parks or preserves where regulation of wildlife populations through conventional means, such as hunting, translocation, culling, or habitat modification has not been effective or feasible, or is precluded because of human opposition. A growing interest in nonlethal methods for population control of nuisance or damaging wildlife species has fostered research in wildlife contraception. Because fertility control acts by reducing birth rates, rather than by increasing mortality rates, it is perceived by the public as being more humane and morally acceptable than conventional population control methods.

Beginning in 1991, the USDA Wildlife Service's National Wildlife Research Center (NWRC) began research into wildlife contraception, with the goal of developing contraceptive products that are practical to use, safe for the treated animal, and present little risk to humans, nontarget animals, and the environment. Working cooperatively with Innolytics, LLC, OvoControl G™ was recently registered for reducing the hatchability of Canada goose eggs. Another product, DiazaCon™, is being tested for field efficacy in monk parakeets, and should begin the registration process within the year. A third product developed by the NWRC, the single-shot GonaCon™ Immunocontraceptive Vaccine, is poised to begin the registration process. This talk will focus on GonaCon™.

Since 1995, NWRC scientists have been working to develop a single-shot, multiyear contraceptive for white-tailed deer. This research has resulted in the development of GonaCon™, a new gonadotropin-releasing hormone (GnRH) immunocontraceptive vaccine. The new vaccine has a APHIS/USDA patent pending status. The single-shot,

Session III: What's New in Contraceptive Vaccines?

multiyear injectable vaccine stimulates the production of antibodies that bind to the GnRH hormone in an animal's body, reducing GnRH's ability to stimulate the release of the sex hormones (e.g., estrogen, progesterone, and testosterone). All sexual activity is decreased, and animals remain in a non-reproductive state as long as a sufficient level of antibody activity is present.

To make the GonaCon™ vaccine, multimers of a synthetic GnRH are coupled to a limpet hemocyanin (either keyhole limpet hemocyanin–KLH or blue protein). This is combined with an adjuvant developed at the NWRC (AdjuVac) that enhances immunogenicity. The AdjuVac is a modification of the USDA-licensed Johnne's disease vaccine Mycopar™ that contains small amounts of killed *Mycobacterium avium*. GnRH is a decapeptide produced in the hypothalamus and carried to the anterior pituitary gland, where it results in release of FSH and LH. In males, LH and FSH are required for testosterone production by the testes and for spermatogenesis. In females, LH and FSH are required for estrogen production, follicle formation, and ovulation. When linked to the large, foreign limpet protein, the resulting GnRH molecule looks like a giant new protein that the animal's immune system has never encountered. As a result, when GonaCon™ is injected into an animal, the animal's immune response makes antibodies to both the limpet protein and to the animal's own hypothalamic GnRH. The antibodies inhibit GnRH interaction with receptors on pituitary gonadotrope cells, and prevent the normal cascade of hormone secretion that is required for gamete production.

Effective long-term immunity normally requires a prime and a boost immunization, followed by periodic additional boosting to maintain immunity. For wildlife species, repeated vaccination is not feasible because they cannot normally be captured more than once. The GonaCon™ vaccine induces a long-lasting contraceptive response with a single injection. A single shot can successfully keep female mammals infertile for one to four years without boosting, and infertility is reversible over time as antibody levels decline. Multiple injections increase the longevity of the vaccine. GnRH is an ideal candidate for immunocontraception of stray animals such as cats and dogs because it is effective in both males and females. GnRH is not species or sex specific, and mammalian GnRH is effective in reducing fertility in most mammals. GonaCon™ has been shown to produce high GnRH antibodies and prevent pregnancy in several species: deer, wild rats, squirrels, cats, dogs, domestic and feral pigs, rabbits, coyotes, wild horses, and bison, following a single dose.

Adverse health effects associated with GonaCon™ are minimal. In field and pen studies, deer showed little evidence of inflammation at injection sites, and blood chemistry was similar among treatment and control groups. Vaccinated animals showed reduced plasma progesterone concentrations, altered estrus behavior, contraception, and reduced fawning rates, all of which were expected. Infertility lasted up to two years without a booster injection and necropsies of recently vaccinated deer showed normal ovaries. To further evaluate toxicity and safety of GonaCon™, a 20-week study was conducted with white-tailed deer where does were given either a single injection of saline, a single injection of GonaCon™ or three injections of GonaCon™ at two-week intervals per dose. Blood samples were taken at intervals during the study and all deer were euthanized and

Session III: What's New in Contraceptive Vaccines?

evaluated at necropsy at 20 weeks. There were no significant contraindications or toxic effects associated with GonaCon™.

There is no danger associated with humans or wildlife eating wildlife that have been vaccinated with GonaCon™. As with other vaccines, such as those used with livestock, both the vaccine and the antibodies produced are proteins. Once ingested, they are broken down by stomach acids and enzymes. The Food and Drug Administration (FDA) determined there would be little risk to humans if meat from vaccinated deer was consumed; the FDA approved the slaughter of pigs vaccinated with GonaCon.

Between 1996 and 2006, the regulatory agency responsible for wildlife contraceptives was the Food and Drug Administration, Center for Veterinary Medicine (CVM). During that period, the NWRC attempted to fulfill CVM's regulatory requirements but it became clear that wildlife contraceptives were incompatible with CVM's regulatory process. The regulatory authority for contraceptives for wildlife and feral animals has recently been moved from the FDA to the Environmental Protection Agency (EPA). The EPA will assume regulatory authority of contraceptives used for wildlife and feral animals. The CVM will retain authority over all uses in captive animals, including livestock, companion animals, and zoo animals.

The NWRC has conducted numerous pen trials and is currently conducting field effectiveness studies with white-tailed deer in two fenced sites in Maryland and New Jersey. Preliminary field data show the vaccine to be 70-85% effective the first year. Pen studies indicate about 70% effectiveness in the second year. The pen and field effectiveness data gathered from NWRC studies will be submitted to the EPA this fall, along with other required data, to obtain a registration for use of GonaCon™ in managing cervid (deer and elk) populations in areas where other management techniques, such as hunting, cannot be used or are socially unacceptable. A registration for GonaCon™ could be granted to USDA as early as fall 2007.

NWRC scientists anticipate that GonaCon™ will be registered as a "Restricted Use" product, probably for use by state or federal wildlife or natural resource management personnel or persons working under their authority. GonaCon™ users will also need to follow state authorization processes. GonaCon™ will not replace other management tools and its use alone cannot rapidly reduce overabundant deer populations to healthy levels. Instead, it is a tool to be used in conjunction with other wildlife management methods. The vaccine can be used to help manage overabundant deer herds in urban and residential areas where other management methods, such as hunting, are not always an option.

Session III: What's New in Contraceptive Vaccines?

Selected References

Killian, G., L. Miller, N. K. Diehl, J. Rhyan, and D. Thain. 2004. "Evaluation of Three Contraceptive Approaches for Population Control of Wild Horses." *Proceedings of the Vertebrate Pest Conference* 21:263-268.

Killian, G., L. Miller, J. Rhyan, and H. Doten. 2006. "Immunocontraception of Florida Feral Swine with a Single-Dose GnRH Vaccine." *American Journal of Reproductive Immunology* 55:378-384.

Killian, G., D. Wagner, and L. Miller. 2005. "Observations on the Use of the GnRH Vaccine GonaCon™ in Male White-Tailed Deer (*Odocoileus virginianus*)." *Proceedings of the Wildlife Damage Management Conference* 11:256-263.

Miller, L. A., B. E. Johns, and G. J. Killian. 2000. "Immunocontraception of White-Tailed Deer with GnRH Vaccine." 2000. *American Journal of Reproductive Immunology* 44: 266-274.

Miller, L. A., J. Rhyan, G. Killian 2004. "Evaluation of GnRH Contraceptive Vaccine Using Domestic Swine as a Model for Feral Hogs." *Proceedings of the 10th Wildlife Damage Management Conference* 10:120-127.

Miller, L. A., J. Rhyan, and G. Killian. 2004. "GonaCon™, a Versatile GnRH Contraceptive for a Large Variety of Pest Animal Problems." *Proceedings of the Vertebrate Pest Conference* 21:269-273.

Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

By Dr. Kathy Fagerstone

GnRH Contraceptive Vaccine: Mechanism of Action and Applications

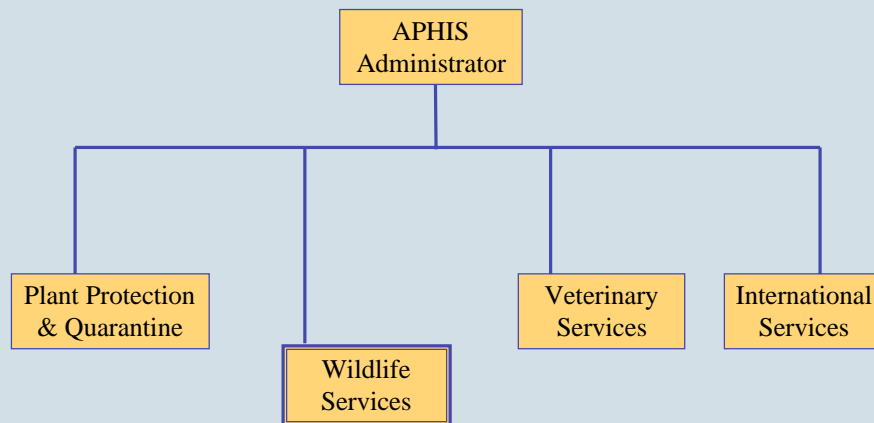
Kathleen A. Fagerstone and Lowell A. Miller
USDA/APHIS/Wildlife Services
National Wildlife Research Center



United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

USDA Animal & Plant Health Inspection Service



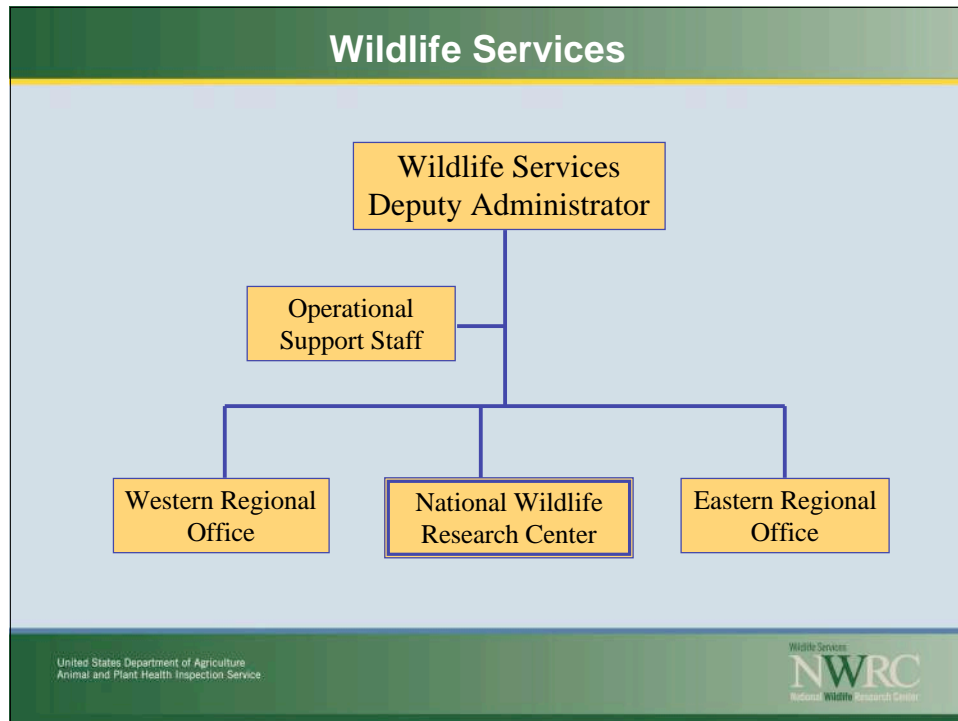
United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

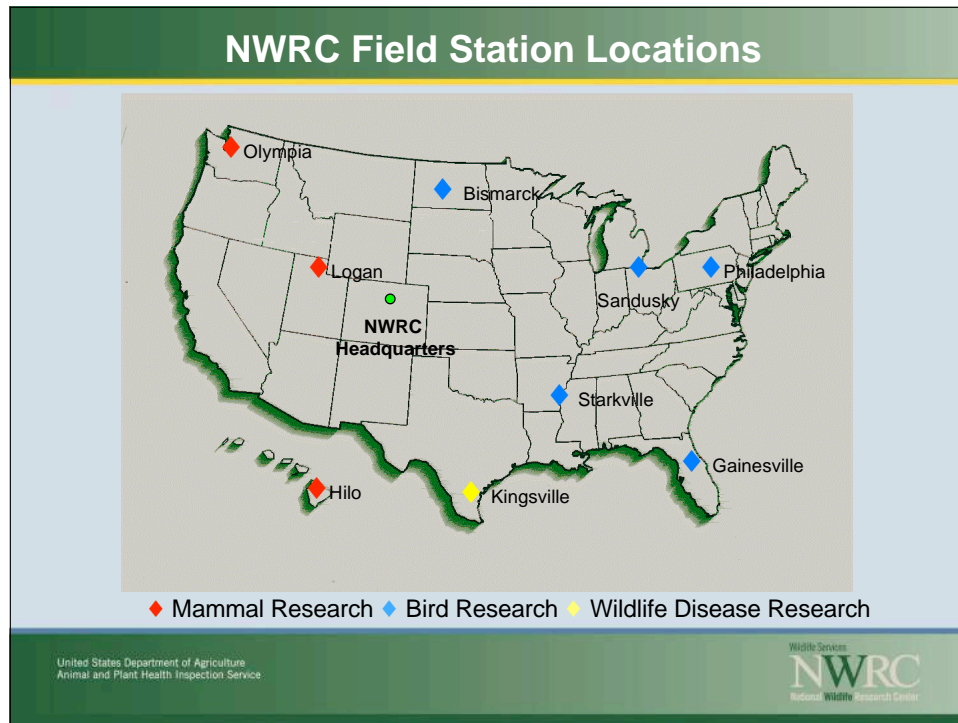
By Dr. Kathy Fagerstone



Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

By Dr. Kathy Fagerstone



Why is the USDA Interested in Fertility Control for Wildlife?

- **U.S. Has Many Overabundant Native Wildlife Species**
 - White-tailed deer
 - Coyotes
 - Crows
 - Feral pigs
 - Canada geese
 - Gulls and cormorants
- **And Invasive Species**
 - Rats
 - Brown tree snakes
 - Pigeons
- **Shift in Public Attitudes Away from Traditional Management Options**
 - Increasing Urbanization Prevents Use of Hunting, Trapping, or Toxicants

United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

By Dr. Kathy Fagerstone

Zoo and Companion Animal Contraceptive Methods

- **Physical (castration, ovariectomy, tubal ligation)**
- **Steroids/hormones**
 - Advantages
 - Can be fed orally or implanted
 - Disadvantages
 - Effective for a short period-- Need repetitive applications
 - Can cause hazards to animals consuming treated animals



United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Wildlife Contraceptive Methods

American Association of Wildlife Veterinarians

RESOLUTION

- Fertility control considered acceptable if the following conditions are met:
 - No effect on health of target species and humans
 - Risk assessment completed for nontarget species
 - Application limited to site-specific populations
 - Effects on population dynamics evaluated
 - Evaluated by wildlife and regulatory agencies
 - Costs are borne by organizations or public that benefit from the program

United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

By Dr. Kathy Fagerstone

NWRC Wildlife Contraceptive Methods

- **Chemicals**
 - **Nicarbazin**
 - Prevents egg hatch
 - **OvoControl G®** registered for urban Canada geese
 - **OvoControl R®** for pigeons soon
 - **DiazaCon**
 - Cholesterol inhibition
 - Monk parakeets




United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

NWRC Wildlife Contraceptive Methods

- **Immunocontraceptive Vaccines**
 - Porcine zona pellucida (PZP)
 - Gonadotrophin Releasing Hormone (GnRH)



United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

By Dr. Kathy Fagerstone

How Do Immunocontraceptives Function?

- Immunocontraception vaccines are directed against “self” reproductive hormones or proteins, to which the recipient is immunologically tolerant.
- The animal’s hormones are made “non-self” by coupling them to a protein that is foreign to the animal.
- The animal makes antibodies to the foreign and the “self” reproductive hormones or proteins.
- The “antiself” antibodies circulate in the target animal and interfere with the biological activity of that hormone or protein.
- Contraception continues as long as the antibody level is sufficiently high to bind the circulating hormone, usually one to four years.

United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

GnRH Immunocontraception

- GnRH is a small peptide hormone secreted from the hypothalamus in the brain that controls release of FSH and LH from the pituitary.
- GnRH is made foreign by coupling it to a limpet hemocyanin protein.
- Antibodies to GnRH interfere with its biological action and reduce secretion of FSH and LH.

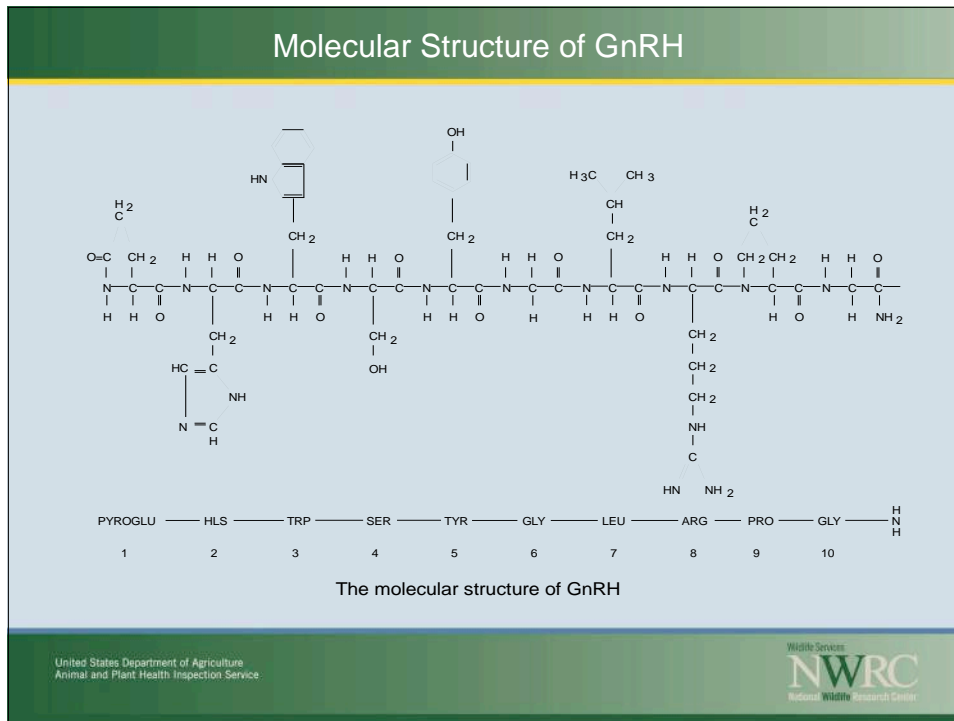
United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

By Dr. Kathy Fagerstone



Mollusk (Limpet) Hemocyanin

- Hemocyanin is an oxygen carrier protein
- Purified from hemolymph: 5-8 million MW
- Each subunit contains 2 copper atoms
- Oxygenated copper provides the blue color



Giant Keyhole Limpet
KLH

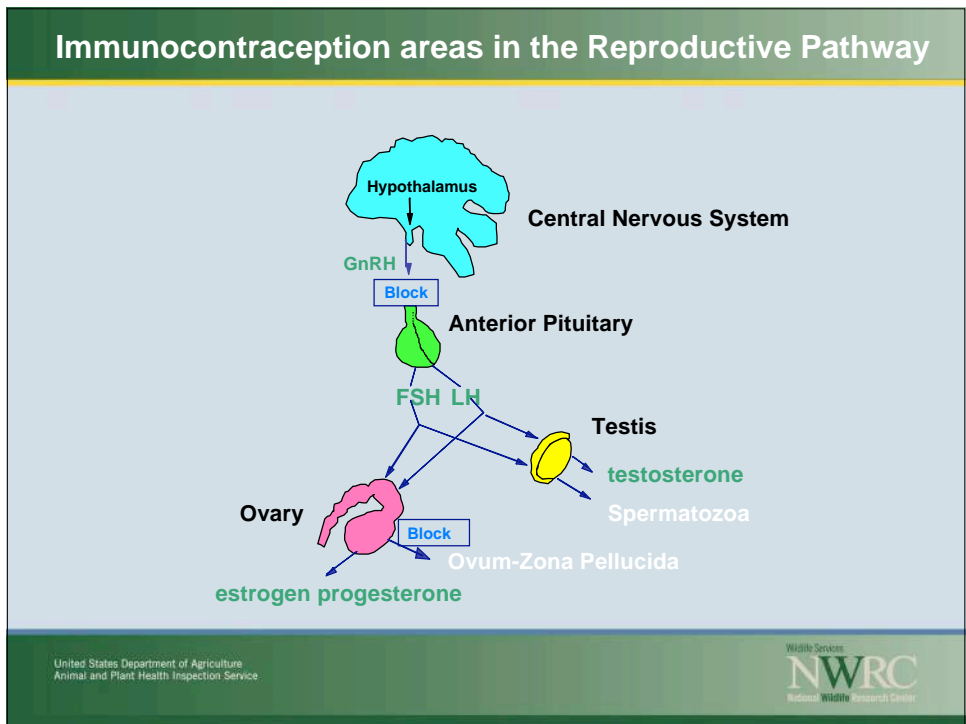
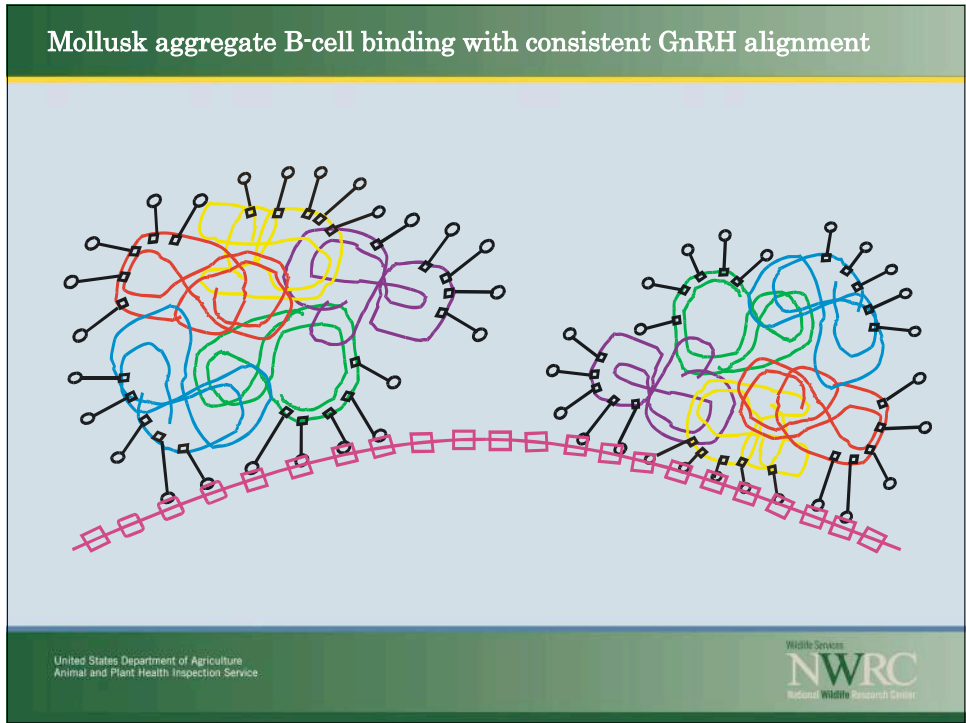
United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

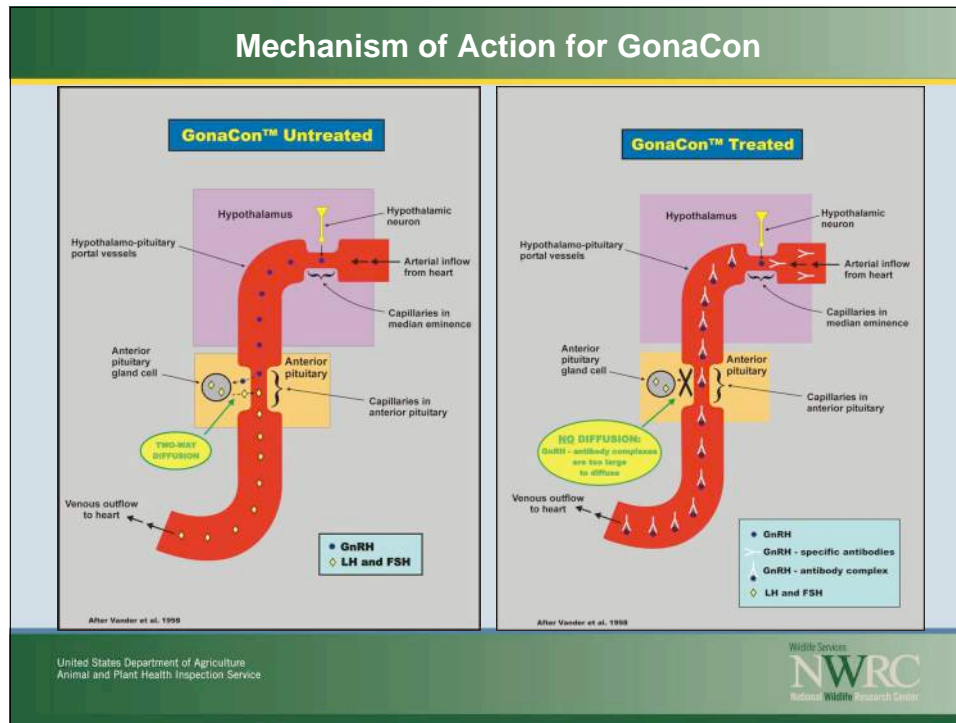
By Dr. Kathy Fagerstone



Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

By Dr. Kathy Fagerstone



Mechanism of Single Shot

- Long-lasting immune response depends on retained antigen
- Macrophage attempts to destroy the antigen
- Follicular Dendritic Cells (FDC) protect the antigen
- FDC protects antigen through bound immune complexes in draining lymph node

Follicular Dendritic Cell

United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

By Dr. Kathy Fagerstone

Development of a New Adjuvant to Replace Freund's

Reason FDA expressed concerns about potential of Freund's adjuvant to cause cancer and granulomas at injection sites.

Solution **AdjuVac™**: Modification of Mycopar, a licensed Johne's vaccine used in cattle. AdjuVac contains a greatly reduced amount of *Mycobacterium avium*.

AdjuVac™ is being sold through USDA's Pocatello Supply Depot.



United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

GonaCon™ Vaccine Developed at NWRC

- GonaCon™ (GnRH vaccine combined with adjuvant)
- USDA patent pending for USA and Europe
- Effective as a single or dual injection
- Reduces sexual activity in males and females; however, effect is longer lasting in females
- The effect is reversible in most cases



United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

By Dr. Kathy Fagerstone

GnRH has been tested in the following species:

- White-Tailed Deer
- Norway Rats
- California Ground Squirrels
- Wild Horses
- Bison
- Domestic and Feral Pigs
- Feral Dogs
- Feral Cats



United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Contraceptive Use For Rodents

Norway Rat Testes



California Ground Squirrel

Berkeley, California, Parks and Beaches

- Squirrels Causing Nuisance and Safety Problems
- Use of Traps and Toxicants Illegal
- Funded by Alameda County Vector Control
 - Paid for use of GnRH Vaccine
- Reduced population to acceptable level



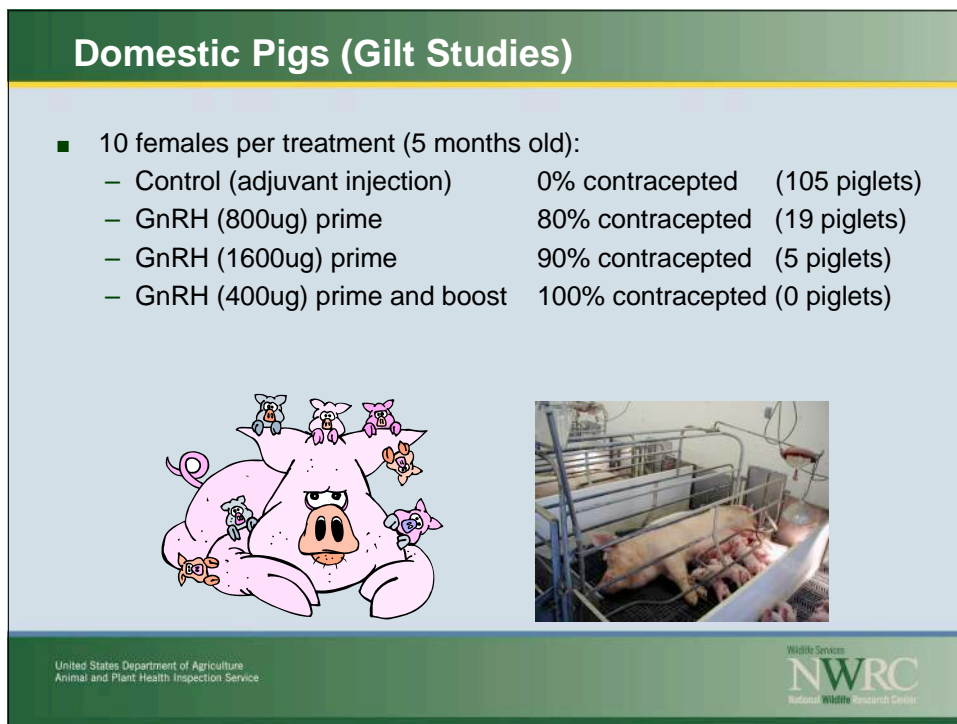
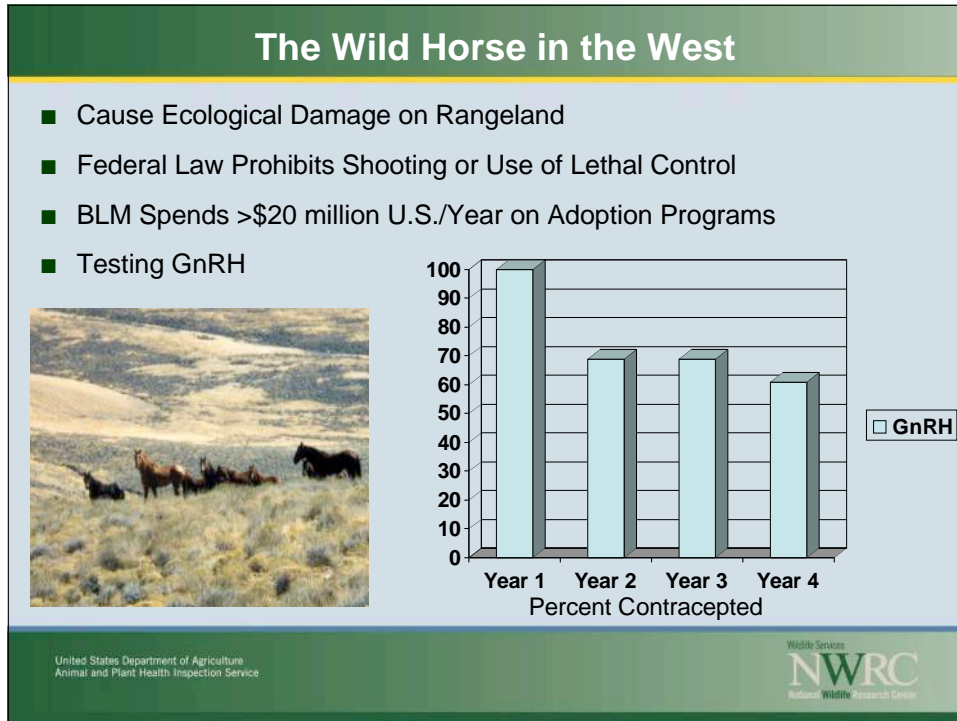
United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

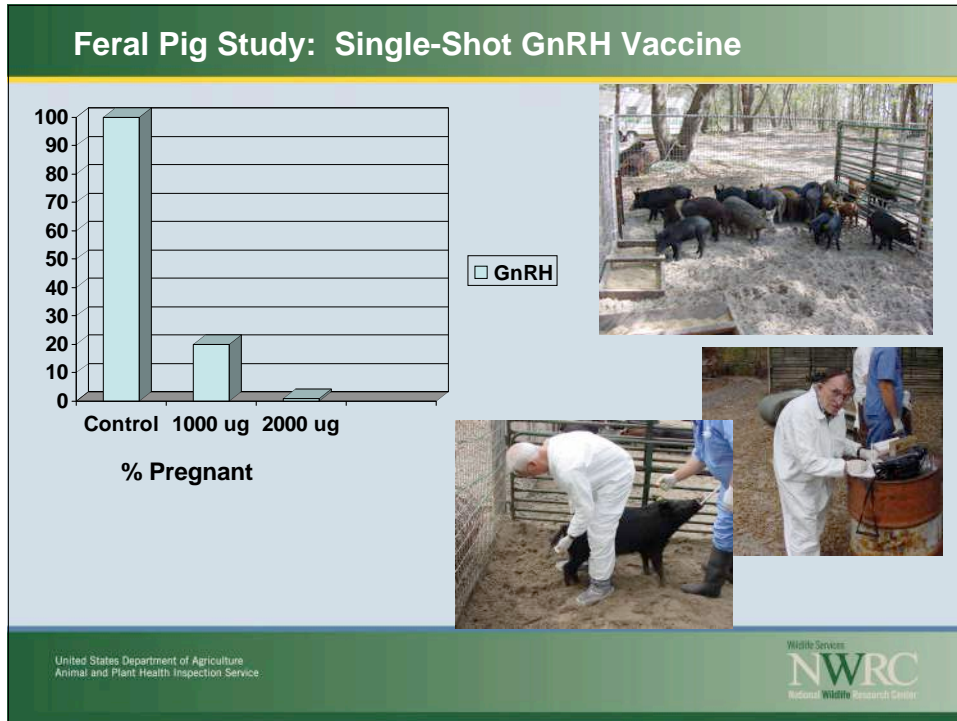
By Dr. Kathy Fagerstone



Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

By Dr. Kathy Fagerstone



Bison Study: Idaho Fish and Game Research Facility

Treatment	Year 1	Year 2
(Injection date 6/02)		
•Control	5/5 preg/5 calves	5/5 preg/5 calves
•GonaCon	4/6 preg/4 calves	0/6 preg/0 calves

■ GnRH given in the last trimester of pregnancy in 4/6 bison; all 4 delivered normal calves between 6/20 and 7/26/02

United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Session III: What's New in Contraceptive Vaccines?




Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

By Dr. Kathy Fagerstone

Maryland White-Tailed Deer Field Efficacy Study with GonaCon

NWRC and Wildlife Services Operations Cooperative Study

- Treated in July 2004
- 2005 Results
 - Control (N=13)
 - 11/13 (85%) bore at least one fawn
 - Treated Deer (N=26)
 - 3/26 (11.5%) bore at least one fawn
- 2006 Results
 - Control (N=10)
 - 10/10 (100%) bore at least one fawn
 - Treated (N=19)
 - 10/19 (53%) bore at least one fawn



United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Does GonaCon meet criteria of a good contraceptive?

■ Effective	100% effective with 2 shots 80-100% effective with 1 shot
■ Long-lasting	Multiple years with single shot
■ Reversible	After antibody levels decline
■ Human safety	OK—Allow consumption
■ Target animal safety	OK—No safety effects
■ Nontarget animal safety	OK—Injection precludes hazards
■ Safe delivery system	Injection (hand or dart)
■ Practicality	Single shot
■ Economical	Requires trapping or darting animal

United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Session III: What's New in Contraceptive Vaccines?

Mechanism of GnRH Contraceptive Vaccine-Mediated Infertility and Its Applications

By Dr. Kathy Fagerstone

Summary

- NWRC is pursuing development of a variety of contraceptive products
- GnRH immunocontraceptive vaccine (GonaCon™) is the result of a lengthy, science-based research process
- NWRC has partnered with numerous agencies/organizations to develop and test this vaccine technology
- GonaCon™ has broad application to multiple species and situations
- There is interest in the technology by state, federal and international organizations
- WS views this as a complementary, not an alternative, tool to current wildlife management methods
- GonaCon™ may be registered within 1½ to 2 years

United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

Questions ?

United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center